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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The Persian Gulf War resulted in friendly fire casualties among U.S. personnel injured by fragments of depleted uranium (DU) munitions. The demonstrated effectiveness of such weapons makes it likely that they may be used against U.S. forces in future conflicts. Uncertainty about how aggressively to remove fragments of the radioactive, chemically toxic DU has stimulated research into the long-term health consequences of embedded DU fragments. There has been no previous research to determine whether long-term exposure to embedded DU can affect the health of offspring of personnel wounded by DU. This study investigates whether male mice carrying embedded fragments of DU transmit genetic damage to their offspring. We hypothesize that long-term chronic exposure to embedded DU results in paternal transmission of genetic damage to unexposed F1 generation offspring, characterized by increased frequency of in vivo mutations in tissues. During this the first year of this project, we have successfully obtained our institutional animal care approvals, standardized pellet implantation surgical protocols in the mouse, established agreements for use of the proprietary "Big Blue" mouse, contracted for DU and HMTA pellet procurements, begun implantation of DU and HMTA pellets in the mice, and initiated breeding of implanted mice.

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Table of Contents

INTRODUCTION

The Persian Gulf War resulted in a number of friendly fire casualties among U.S. personnel injured by fragments of depleted uranium (DU) munitions. The demonstrated effectiveness of such weapons makes it likely that they may be used against U.S. forces in future conflicts. Uncertainty about how aggressively to remove fragments of the radioactive, chemically toxic DU has stimulated research into the long-term health consequences of embedded DU fragments. There has been no previous research to determine whether long-term exposure to embedded DU can affect the health of offspring of personnel wounded by DU. This study investigates whether male mice carrying embedded fragments of DU transmit genetic damage to their offspring. Other studies have demonstrated that paternal preconceptional exposure to radiation or metal can induce cancer in unexposed offspring. The transgenic mouse model proposed for this study allows us to estimate overall mutation frequency in tissues from progeny of exposed males by following mutations in lacI gene. We hypothesize that long-term chronic exposure to embedded DU results in paternal transmission of genetic damage to unexposed F1 generation offspring, characterized by increased frequency of in vivo mutations in tissues. Transgenerational transmission of genetic damage may occur because of an induction of germ-line genomic instability and/or direct sperm mutations. Results of this study will provide new information about potential health effects of embedded fragments of "non-traditional" metals such as DU will allow an update of existing fragment removal policies.

BODY

This report summarizes accomplishments for the first year of this two-year study. The approved Statement of Work for year 1 is as follows.

- Begin surgical implantation of DU and HMTA pellets
- Initiate and complete breeding of implanted mice with unimplanted females
- Initiate harvesting of tissues from F1 progeny of implanted fathers
- Start analysis of mutation frequency in bone marrow of F1 progeny
- Initiate and complete breeding of neutron-irradiated mice with unirradiated females
- Initiate and complete harvesting of tissues from F1 progeny of neutron-irradiated fathers
- Initiate and complete analysis of mutation frequency in bone marrow of F1 progeny

Initiation of the project was delayed for 4-5 months and subsequent progress was slower than expected for several reasons. First, there were procurement problems with the tungsten pellets. The tungsten pellet procurement problem arose because of our decision to choose a different vendor than originally planned. The change, however, allowed us to obtain superior pellets at less cost, but it also delayed our receiving them. Second, the Institutional Animal Care and Use Committee did not approve the project until June 2002. Once the IACUC approval process was initiated at USAMRMC it was expeditiously completed. Third, the licensing agreement between us and the transgenic mouse provider (Stratagene, Inc., La Jolla, CA) was delayed until all institutional animal use approvals were completed. The licensing agreement was therefore, did not become effective until August 2002. Finally, the acquisition of an experienced technical assistant for the animal breeding process was slower than anticipated.

The delays mean that we were late initiating implantations of both the DU and HMTA pellets and have just recently begun the breeding process. We have initiated the harvesting of tissues from control animals and the analysis of the bone marrow in these animals. To make up for the lost time we are training additional personnel to work on the molecular analyses portion of this study. By adding another technical-molecular biology assistant to help with this project (at no cost to the grant) we expect that we will be able to double the mutagenicity analyses work effort and reduce the time needed to complete this work.

Because we are adjusting future schedules to make up the lost time, we expect to meet our current second year milestones. We therefore do not see a need at this time to request a change in our future milestones.

Aside from the initial delays, the project is otherwise proceeding as planned. We successfully completed a number of important steps that were required for the project to proceed. We designed and established a new animal surgery facility specifically for this project. We have established and standardized a new mouse anesthesia system, evaluated surgical closure techniques, and new approaches for post-operative pain alleviation and prevention of infection. We tested various animal husbandry requirements recently introduced into our institute and initiated traditional ear tagging.

The first delivery of mice for the studies were received in October 2002 and we have initiated the following experiments: 1) DU and HMTA pellet implantation in male transgenic mice; 2) breeding of transgenic mice (control); 3) breeding of neutron-irradiated mice; and 4) completed neutron radiation exposures.

All mice have been closely monitored from the time they arrived at the Institute. Monitoring includes weekly bodyweight measurements, routine handling of the animals to reduce stress during manipulations, and basic assessments of health.

In parallel with the animal surgeries, we have initiated testing and standardization of our inductively coupled plasma mass spectrometer (ICPMS) instrument for the various metal measurements required for this study. Metals to be measured are uranium (including constituent uranium isotopes), tantalum, tungsten, nickel, and cobalt. We have prior, extensive experience with uranium measurements. Sensitive measurement of metal distributions to tissues is an integral part of validating any abnormalities that we might see in the pellet-implanted mice or their offspring.

KEY RESEARCH ACCOMPLISHMENTS

None to date.

REPORTABLE OUTCOMES

None to date.

CONCLUSIONS

Even though the planned initiation of our experiments was delayed by several months, the project is proceeding as planned. We expect that the lost months will be made up by the end of year two by the addition of personnel who will accelerate the molecular analyses. Experimental methods to be used have been tested and standardized. We have encountered no experimental problems to date that require modification of our original plans and goals.